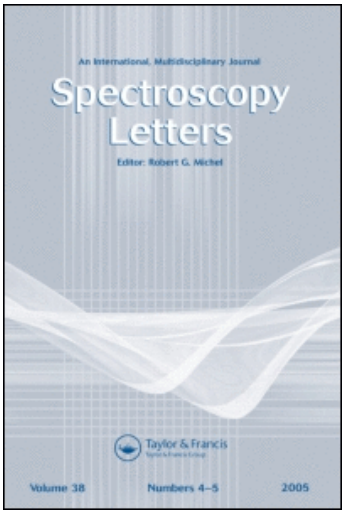

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Spectrophotometric Determination of Certain Cephalosporins Using Ferrihydroxamate Method

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SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN CEPHALOSPORINS USING FERRIHYDROXAMATE METHOD

Key words: Cephalexin, Cefixime, Ceftriaxone,Cefotaxime, Ferrihydroxamate method, Dosage forms assay

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ABSTRACT

Cephalexin, cefixime, ceftriaxone and cefotaxime were determined spectrophotometrically in the pure form and in pharmaceutical formulations by using ferrihydroxamate method. Reaction optimization with respect to reaction time and temperature has been investigated. Influence of the presence of ester functional group on the determination of cephalosporins as ß-lactams under conditions optimized was evaluated. Using cefotaxime sodiume as model drug with ester functional group, it was shown that proposed method gives equally acurate and precise results even in the presence of ester functional group.

INTRODUCTION

Cephalosporins are effective against certain bacterial species through inhibition of cell wall synthesis by competitive hindance of transpeptidation reaction (1). The chemistry of cephalosporins has been widely explored because of their extensive medicinal applications.

The visible spectrophotometric methods for cephalosporins are based on their interaction with different reagents, such as imidazole in the presence of mercury (II) (2), ninhydrine (3), N.N-dimethyl-D-phenylendiamine and ferric ions (4), ammonium vanadate (5), phenol with sodium nitroprusside and hypochlorite (6), 4--dimethylaminocinnam aldehyde (7), 3-bromo-4,4-dimethyl-2-oxazolidinone (8), O-hydroxyhydroquinone phtalein (9), paramolybdate anion (10), sodium nitrite (11), chloranilic acid (12), molybdophosporic acid (13), copper (II) acetate (14), oxidized heamatoxylin (15), 2-nitro-phenyl-hydrazine hydrochloride (16), mercurochrome (17). Derivative spectrophotometric assay of cephalosporins in the presence of their alkaline or acid degradation products have been reported (18, 19). High-presure liquid chromatography for the quantitative determination is also reported (20, 21). Another quantitative methods described for cephalosporins determination include polarography (22), differential pulse polarography (23), gas chromatography (24) and ion-pair reversed-phase chromatography (25).

Hydroxamic acid formation has been employed in the analysis of various carboxylic acid derivatives. Typically, the substrate reacts with an alkaline hydroxylamine solution, acidified, and than complexed with ferric ion to form a color complex. Compounds containing the β–lactam moiety, e.q. cephalosporins, also have been analysed by the hydroxylamine-perchlorate method (26), and nickle catalyzed hydroxiaminolysis (27). The kinetics and the mechanism of hydroxamic acid formation have been studied previously (28) as well as the stability of complexes which these acids form with iron (III) (29).

This paper concerns the investigation of reaction conditions for conversion of certain cephalosporins (cephalexin, cefixime, ceftriaxone, cefotaxime) as ß-lactams into the corresponding hydroxamic acids which formed colored complexes with iron (III) . Under the optimum conditions the proposed spectrophotometric method was evaluated and applied to dosage form assay.

EXPERIMENTAL

Apparatus - A Specord M40, (Carl-Zeiss, Jena, Germany) spectrophotometer equiped with 10 mm glass cells was used.

Reagents and solvents- **Cephalexin monohydrate**-(7R)-3-Methyl-7-(α-D-phenylglycylamino)-3-cephem-4-carboxylic acid, **cefixime** - (Z)-7-[2-(2-Aminothiazol--4yl)-2-(carboxymethoxyimino) acetamido]-3-vinyl-3-cephem-4-carboxylic acid., **ceftriaxone disodium** -(Z)-7-[2-(2-Aminothiazol-4yl)-2-methoxyiminoacetamido]-3-[(2.5-dihydro-6-hydroxy-2-methyl-5-oxo-1.2.4-triazin-3yl)thiomethyl]-3-cephem-4-carboxylic acid and **cefotaxime** (7R)-3-acetoxymethyl-7-[(Z)-2-(2-aminothiazol-4yl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate,were used as working standards; hydroxylammonium chloride (Merck); iron (III) perchlorate (Fluka); sodium hydroxide (Zorka, Yugoslavia), 70% perchloric acid (Merck); methanol and absolute ethanol (Merck) were used. All solvents and reagents were of analytical grade of purity.

Dosage forms - Cefalexin® capsules (500 mg), Panfarma; Oroken® granules (40 mg), Pharmuka; Longaceph® ampules (1g) , ICN Galenika and Tolycar® ampules (1g), Jugoremedia.

Solutions

Reagent A: Equal volumes of 12.5% methanolic hydroxylammonium chloride solution and 12.5% methanolic sodium hydroxide solution were mixed and filtered. The prepared solution was stable for 4 h.

Reagent B: (Stock solution); Accurately weighed 5g of iron (III) perchlorate was dissolved in the mixture of 10 ml perchloric acid and 10 ml of water. The prepared solution was diluted with absolute ethanol up to 100 ml, with cooling.

Reagent B_1: 1.2 ml of perchloric acid was added to 4 ml of the reagent B with cooling. The prepared solution was diluted to 100 ml with absolute ethanol.

Standard solutons 1,2,3 and 4: Accurately weighted 20.0 mg of cephalexin monohydrate, cefixime,ceftriaxone disodium and cefotaxime sodium, respectively were transferred to a 10 ml calibrated flasks and dissolved in methanol up to 10 ml.

Standard solution 1a: Accurately weighted 40.0 mg of cephalexin monohydrate was transferred to a 10 ml calibrated flask and dissolved in methanol up to 10 ml.

Sample solution (I): A quantity of Cefalexin® capsules containing 500 mg of cephalexin monohydrate was transferred to 100 ml calibrated flask, and dissolved

up to the mark wih methanol. An aliquot of 4 ml of this solution was then diluted with methanol in 10 ml volumetric flask.

Sample solution (II): A quantity of Oroken® foil packets containing 40 mg of cefixime was transferred to a 10 ml calibrated flasks, and dissolved up to 10 ml with methanol, mixed and filtered.

Sample solution (III): A quantity of Longaceph® ampules containing 1g of ceftriaxone was transferred to 100 ml calibrated flasks and dissolved up to the mark wih methanol. An aliquot of 2 ml of this solution was then diluted with methanol in 10 ml volumetric flask.

Sample solution (IV): A quantity of Tolycar® ampules containing 1g of cefotaxime was transferred to 100 ml calibrated flasks and dissolved up to the mark with methanol. An aliquot of 2 ml of this solution was then diluted with methanol in 10 ml volumetric flask.

General procedure

Equal volumes of standard solutions 1, 2, 3 and 4 (0.5 ml) were transferred to 5 ml volumetric flasks. After adding 0.3 ml of reagent A to each, the volumetric flasks were stopped and kept for 45 minutes at room temperature followed by dilution to volume with reagent B_1 . The absorbances were measured at 525 nm against the blank, which was prepared as described above but without standard solutions.

The same procedure was carried out for sample solutions to determine cephalosporines from the investigated dosage forms.

Calibration curves

Nine samples containing 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml of each standard solutions were transferred to 5 ml calibrated flasks. After adding to each sample 0.3 ml of reagent A, the reaction mixtures were stopped and kept for 45 minutes at room temperature, followed by dilution up to the mark with reagent B_1. Absorbances were measured at 525 nm against blank.

Stoichiometric relationship

For Bent-French method which was employed, equal volumes of standard solution 1a (1 ml) were transferred to six 5 ml calibrated volumetric flasks, 0.3 ml of reagent

A was added to each of them. After 45 minutes storage at room temperature, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml of reagent B were added to each reaction mixture, respectively, and diluted up to the mark with blank, prepared as reagent B, but without iron (III) perchlorate. Absorbances were measured at 525 nm against blank.

Effect of temperature

Twelve samples containing equal volumes (0.6 ml) of each standard solution of cephalosporins were transferred to 5 ml calibrated flasks. After adding 0.3 ml of reagent A to each, the volumetric flasks were stopped and heated for 20 minutes at 25, 30, 40, 50 and 60°C, respectively. After cooling, the solutions were diluted to volume with reagent B_1. The absorbances were measured at 525 nm against the blank. The same procedure was applied to twelve further samples with heating time prolonged up to 40 minutes.

Effect of reaction time

Nine samples containing equal volumes (0.6 ml) of each standard solution of cephalosporins were transferred to 5 ml calibrated flasks. Reagent A (0.3 ml) was added to each sample. The reaction mixtures were diluted up to the mark with reagent B after 5, 15, 25, 35, 40, 50, 55 and 65 minutes, respectively. Absorbances were measured at 525 nm against blank.

RESULTS AND DISCUSSION

To obtain optimum conditions for cephalosporins determination, the following parameters were studied:

1) Effect of temperature

A study was done to find the optimum temperature of hydroxyaminolysis of cephalosporins leading to maximum intensity of color for a given concentration of cephalosporin. It was found that the quantitative formation of RHA-Fe(III) complex was obtained at room temperature, which is presented in Figure 1.

2) Effect of reaction time

Investigation revealed that the optimum reaction time of hydroxyaminolysis of cephalosporins at room temperature is 45 minutes, which is presented in Figure 2.

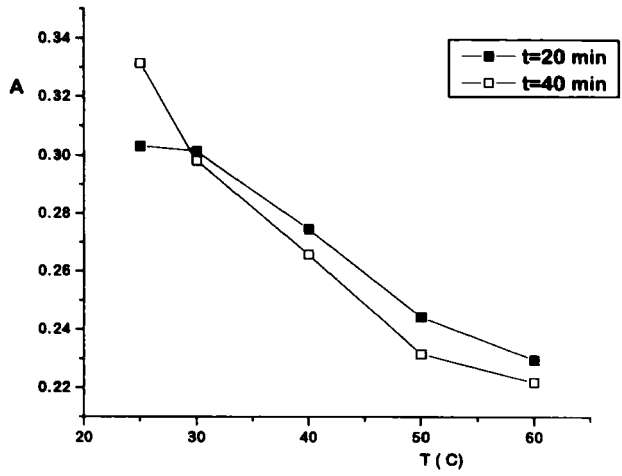

FIG.1. The effect of the temperature on the color intensity of RHA-Fe(III) complex ($C_{cephalexin\ monohydrate}$ = $6.57 \cdot 10^{-4}$mol/l)

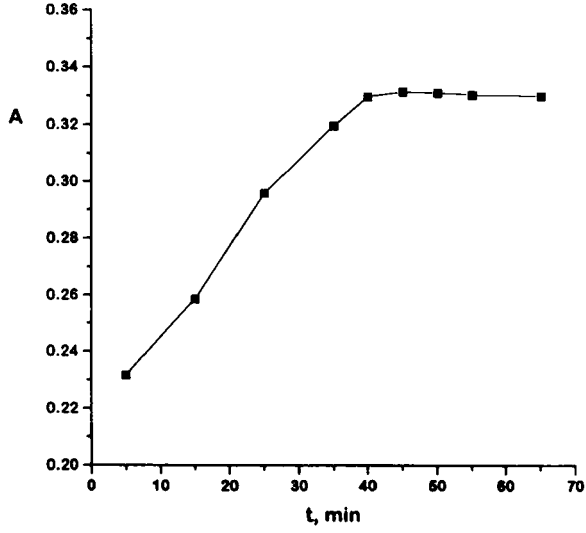

FIG.2. The effect of the reaction time on the color intensity of RHA-Fe(III) complex ($C_{cephalexin\ monohydrate}$ = $6.57 \cdot 10^{-4}$mol/l)

As the hydroxiaminolysis of cephalosporines takes part at room temperature, β-lactam can be determined in the presence of ester functional group, which undergo the hydroxiaminolysis at higher temperatures.

Considering the determination of cefotaxime, which includes both β-lactam and ester functional groups, it was shown by quantitative determination of cefotaxime, that esther group did not react at room temperature, thus, not influencing the determination of cefotaxime. Higher absorbance values obtained at higher temperatures, indicated that under such conditions, ester group reacted, too.

The investigation of Oroken® granules showed that presence of hydroxypropylcellulose, does not influence the quantitative determination of cefixime.

The graph of absorbance vs the concentration of cephalexin was linear in the range from 80-320 μg ml^{-1} (2.2×10^{-4} - 8.75×10^{-4} mol l^{-1}). Beer's law for cephalexin was given by the equation: y=0.0007+518.31x; r=0.9993 and molar absorptivity 0.51×10^3 l $mol^{-1}cm^{-1}$. The intercept a=0.0007 was statistically insignificant. The detection limit for cephalexin was 40 μgml^{-1} (1.1×10^{-4} mol l^{-1}).

The graph of absorbance vs the concentration of cefixime was linear in the range from 80-320 μg ml^{-1} (1.7×10^{-4} - 7.2×10^{-4} mol l^{-1}). Beer's law was given by the equation: y= 0.0094+501.4x; r=0.9999 and molar absorptivity 0.54×10^3 l mol^{-1} cm^{-1}. The intercept a=0.0094 was statistically insignificant, the limit of detection of cefixime was 48 μgml^{-1} (1.05×10^{-4} mol l^{-1}).

The graph of absorbance vs concentration of ceftriaxone was linear in the range from 80-320 μgml^{-1} (1.2×10^{-4} - 4.9×10^{-4} mol l^{-1}). Beer's law was given by the equation: y=-0.009+1088.25x; r=0.9997 and molar absorptivity 1.05×10^3 l $mol^{-1}cm^{-1}$. The intercept a= -0.009 was statistically insignificant, the limit of detection of ceftriaxone was 72 μgml^{-1} (1.1×10^{-4} mol l^{-1}).

The graph of absorbance vs concentration of cefotaxime was linear in the range from 80-320 μg ml^{-1} (1.7×10^{-4} - 6.7×10^{-4} mol l^{-1}). Beer's law was given by the equation: y=-0.0015+589.31x; r=0.9996 and molar absorptivity 0.6×10^3 l mol^{-1}

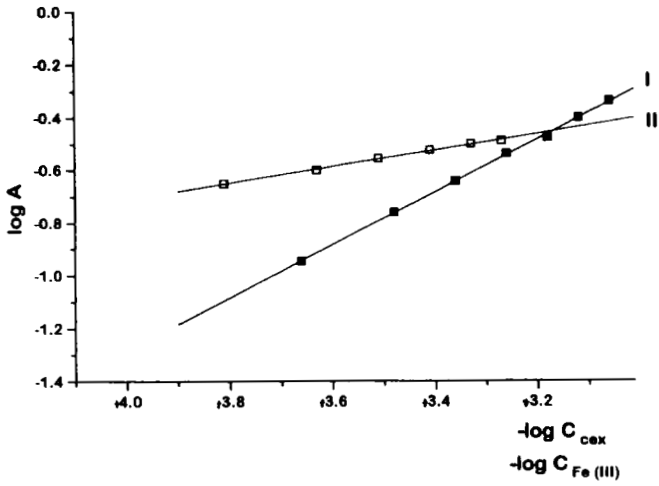

FIG.3. The dependence of the logarithm of absorbance value on the negative logarithm of cephalexin concentration (curve I) and on the negative logarithm of Fe(III) concentration (curve II).

TABLE 1

Results obtained from Bent-French method for cephalexin

	Coefficient n	$C_{Fe(III)}$ mol l^{-1}	$C_{cephalexin}$ mol l^{-1}	r
curve 1	1.00	3.02×10^{-3}	2.19×10^{-3} - 8.75×10^{-3}	0.9994
	Coefficient m	$C_{cephalexin}$ mol l^{-1}	$C_{Fe(III)}$ mol l^{-1}	r
curve 2	0.31	2.19×10^{-3}	1.55×10^{-4} - 5.4×10^{-4}	0.9972

cm^{-1}. The intercept a=-0.0015 was statistically insignificant. The limit of detection of cefotaxime was 40 $\mu g ml^{-1}$ (1.0×10^{-4} mol l^{-1}).

The stoichometric composition of the corresponding hydroxamic acid-iron (III) complex was determined by Bent-French method. The dependance of the logarithm of absorbance value on the negative logarithm of cephalexin concentration (curve 1) and on the negative logarithm of iron (III) concentration (curve 2) are presented in Figure 3. The slope values were 1.00 and 0.31, respectively (Table 1).

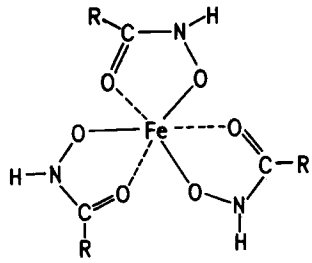

FIG.4. Structure of RHA-Fe(III) complexes

TABLE 2

Statistical evaluation of the precision of the proposed method

Sample	taken (mg ml^{-1})	A (n=6)	RSD(%)
cephalexin	80	0.112	1.68
	240	0.335	0.54
	320	0.458	1.14
cefixime	80	0.100	1.98
	240	0.275	0.65
	320	0.370	1.24
ceftriaxone	80	0.119	1.68
	200	0.318	0.56
	320	0.518	1.21
cefotaxime	80	0.100	1.60
	240	0.294	0.71
	320	0.390	1.05

Bent-French method leads to the conclusion that the stoichometric composition of the complex formed between iron(III) and corresponding hydroxamic acid formed by hydroxiaminolysis of cephalosporin is 1:3. In accordance with literature data (30) the proposed structure of investigated complexes are presented at Fig.4.

The precision of the proposed method was checked at three different concentrations of cephalexin, cefixime, ceftriaxone and cefotaxime. The obtained results with statistical parameters are presented in Table 2.

TABLE 3

Dosage forms assay

dosage form	taken (mg)	found (mg)	RSD(%)
Cefalexin®	500	501.9	0.62
Oroken®	40	41.69	1.85
Longaceph®	1000	1013.98	1.87
Tolycar®	1000	1008.17	1.96

Table 3 shows the results obtained from the determination of cephalexin, cefixime, ceftriaxone and cefotaxime in dosage forms by means of the proposed method. The obtained results and statistical parameters for the determination of cephalexin, cefixime, ceftriaxone and cefotaxime demonstrate that the proposed procedure was suitable for application in routine control of cephalosporins in dosage forms.

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